# Dynamics of Tumor Suppressor p53 along DNA Revealed by High-speed Single-molecule Fluorescence Microscopy

（別紙様式５）

# 論　文　内　容　要　旨

（NO．1）

| 氏　　名 | Dwiky Rendra Graha Subekti | 提出年 | 令和２年 |
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| 学位論文の<br>題　　目 | Dynamics of Tumor Suppressor p53 along DNA Revealed by High-speed Single-molecule Fluorescence Microscopy<br>（高速単一分子蛍光顕微鏡法によるがん抑制タンパク質 p53 の DNA 上におけるダイナミクス） | | |

## 論　文　目　次

## 1. General Introduction

DNA binding proteins are involved in various processes that are crucial in maintaining and regulating cellular functions. The biochemical reactions and signaling pathways involved in these processes are triggered and regulated by DNA binding proteins. DNA binding proteins are required to find and bind to their target sites among the enormous length of DNA within a physiologically relevant time scale. The typical size of the target sequence for many DNA binding proteins is 5~30 bp, while the typical size of genomic DNA is $10^6$-$10^9$ bp. The search time for the target sequence of DNA binding proteins is estimated to be within several tens of minutes in vivo. Assuming that a single DNA binding protein searches for its target by repeatedly associating and dissociating with random sites in the genome, the target search time is estimated to be one to two orders of magnitudes slower than the in vivo response time. This gap, also known as “target search problem”, suggests that there may exist a mechanism that allows the acceleration of the target search time for DNA binding proteins. In this thesis, I investigated the physical mechanisms involved in the target search of DNA binding proteins.

To investigate the target search mechanism of DNA binding proteins, I chose p53 as a model protein. p53 monomer is 393-residue long, which is composed of disordered N-terminal (NT) domain (residues 1–95), structured core domain (residues 95–293), disordered flexible linker (residues 293–326), structured tetramerization (Tet) domain (residues 326–357), and disordered C-terminal (CT) domain (residues 357–393). The core domain is folded with an immunoglobulin-like $\beta$-sandwich structure. The tetramerization domain is responsible for the formation of p53 tetramer, which is the basic functional unit for p53 *in vivo*. The CT domain is highly charged by positively charged residues and interacts with DNA nonspecifically. The linker domain connects

the core domain with the tetramerization domain, and contains one of the nuclear localization signals for the transfer of p53 from cytoplasm to nucleus.

Previous investigations on p53 strongly indicate that the target search dynamics of p53 is controlled by different conformations of p53 during its interaction with DNA. For an example, despite being highly conserved and possible contribution in p53's flexible structure, the roles of the intrinsically disordered region (IDR) connecting the core and tetramerization domains as the linker (IDR linker) in the target search mechanisms are largely uninvestigated. Furthermore, it has been speculated that many transient events occur during the target search that are experimentally unobservable due to insufficient time resolution of the current experimental apparatus. Thus, in this thesis, I would like to; 1) Investigate how the linker affects p53 structure and its binding dynamics, and 2) Explore the currently unobservable target search events and the corresponding structures.

2. Role of IDR Linker in p53 Target Search

I investigated whether the linker region of p53 has roles in the p53-DNA interaction and how it affects the target search. Based on the sequence of the linker region, I first hypothesized that the linker in p53 interacts directly with DNA and regulates the binding to specific and non-specific DNA sequences. Secondly, I also hypothesized that the length of the p53 linker is correlated with the efficiency of the 1D sliding and the target binding by promoting or restricting the movement of the core domain, e.g., elongating the linker may promote the 1D sliding of p53. To prove the hypotheses, I designed several p53 mutants with modulated linkers and analyzed them by using different techniques. To investigate the effect of the modulated linker on the binding to DNA, I determined the equilibrium dissociation constant by fluorescence anisotropy titration. To evaluate the effect of linker modulation on 1D sliding dynamics, I utilized single-molecule fluorescence microscopy coupled with the DNA array technique "DNA garden" and determined the diffusion coefficient for each mutant. I found that while the elongation of the linker decreases the equilibrium dissociation constant of p53 to the non-specific DNA, the 1D diffusion characteristics does not change. In contrast, the substitution of the charged amino acids in the linker to neutral one causes the increase in the equilibrium dissociation constant and the significant change in the 1D diffusion characteristics. The data suggest that the linker interacts directly to DNA and is involved in the 1D diffusion.

3. Sub-millisecond Single-molecule Fluorescence Imaging of p53

I investigated the target search events of p53 that were hidden in the previous investigations due to the insufficient time resolution of typical single-molecule fluorescence microscope. I hypothesized that p53 should exhibit several target search

events that occur in the time scales from microseconds to low milliseconds due to its unique structure. I developed a single-molecule fluorescence microscope with sub-millisecond time resolution and investigated the target search dynamics of p53 along DNA. Using the developed system, I observed new p53 dynamics such as the formation of the transient encounter complex, the jump motion along DNA, and the 1D diffusion at the physiological salt concentrations. I found that more than 90% of the binding events observed by the new single-molecule microscope are that of the transient encounter complex, suggesting that there is a large energy barrier between the transient encounter complex and the stably bound complex. The jumps were observed across the different salt concentrations. While the frequency of the jumps increased at the higher ionic strength, the average jump time that is the time between the dissociation and re-association of p53 to DNA was constant and was several milliseconds. I also observed that the 1D diffusion of coefficient of p53 increased along with the higher salt concentration to the extent correlated with the increase of the jump frequency. The data suggested that p53 in the physiological salt concentrations combines the 1D sliding and the hopping, which is a smaller jump that is indistinguishable from the sliding due to the spatial resolution limit of the current observation. These results showed that p53 exhibits many different events during its binding to and sliding along DNA that are attributed to the different conformations and structures assumed by p53 and the bound DNA.

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論文審査の結果の要旨

配列特異的 DNA 結合タンパク質は、DNA 上の特定のターゲット配列を莫大な DNA 配列のなかから短時間内に選び出し、結合することができる。Subekti 氏は発ガン抑制タンパク質として知られる p53 を研究対象として、p53 の DNA 上におけるターゲット探索機構を調べた。p53 は、細胞に対するストレスにより活性化され、DNA の標的配列に結合することで細胞周期の停止などを制御する重要なタンパク質である。

p53 は複数の構造領域により構成されるタンパク質である。第一の研究プロジェクトにおいて、Subekti 氏は p53 のリンカー領域の特徴を調べた。リンカー領域は、ターゲット配列を認識する複数のコア領域をつなぐ天然変性ドメインである。Subekti 氏は、リンカー部分の長さを変えた変異体や、リンカー部分の荷電アミノ酸を変異させた変異体を作成し、これらの変異体の DNA 上における一次元拡散運動を一分子蛍光顕微鏡により調べた。さらに、これらの変異体の DNA への結合特性も調べた。その結果、リンカー領域が DNA と積極的に相互作用し、コア領域と DNA の相互作用を補助する役割を持つことを見出した。

第二の研究プロジェクトにおいて、Subekti 氏は蛍光顕微鏡を改良し、サブミリ秒の時間分解能による一分子蛍光観察を始めて可能にした。開発した装置を用いることで、DNA 上における新しい p53 のダイナミクスを多数見出した。第一に、p53 が DNA と長寿命の複合体を形作る前に、数ミリ秒程度の短寿命のエンカウンター結合体を形作ることを見出した。第二に、p53 が DNA への結合と解離を繰り返すジャンプ運動を示すことを見出した。第三に、生理的な条件に近い高塩濃度の条件において、p53 の DNA 上における一次元拡散運動が加速することを見出した。これらの結果から、p53 は DNA 上を細かいジャンプを繰り返しながら運動することを推定した。

以上の結論は、Subekti 氏が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって，Subekti 氏提出の博士論文は，博士（理学）の学位論文として合格と認める。